Table 1. Plastic cell numbers and types when different intra-amygdala plasticity components are inactivated.
•Histogram of frequency distribution of plastic cells

Competition among model lateral amygdala principal cells during Pavlovian fear conditioning

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Introduction

The CS responsiveness of principal neurons (PNs) in the dorsal rodent rat lateral amygdala (LAd) is increased after auditory fear conditioning. In the most dorsal part of LAd, PNs display increases in CS responsiveness that last for only a few trials (transiently plastic or TP cells) whereas the more ventrally located PNs show a persistent increases in CS responses, even resisting extinction training (long-term plastic or LP cells; Repa et al.'01). A biophysical 1000-cell model of LAd (Kim et al., 2013a; Fig. 1) successfully replicated the contrasting temporal patterns of increased tone responsiveness displayed by neurons (Fig. 1A) in different parts of LAd. The model also revealed (Kim et al., 2013b) how competition among PNs might occur during the formation of the 'fear memory trace' (Han et al.'07,'09). Here we use the model in Kim et al.(2013) to derive further insights into the formation of this fear memory trace (plastic cells).

Methods

A. Using a slightly revised version of the model in Kim et al. (2013), in the first of experiments, we simulated increases in intrinsic excitability resulting from CREB over-expression by converting fast adapting PNs (type A cells) into slowly adapting ones (type C) in various proportions ranging from 25% to 100%. In the CREB over-expression cases, Winners were PNs that were initially non-plastic but subsequently became plastic, and vice versa for losers.

B. In the second set of experiments, we examined the roles of excitatory and di-synaptic inhibitory connections, and of attributes such as presence of tone, shock, and NM receptors, in the competition among PNs for inclusion in the fear memory trace.

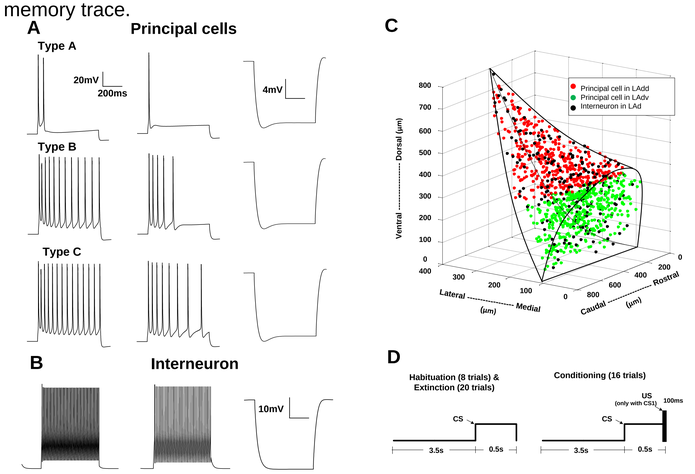

Figure 1. LAd network and training protocol. **A:** Responses of the three types of PNs (A, B and C) to current injections (left: 400 pA; middle: 300 pA; right: -100 pA; duration 600 ms) are similar to those reported in Faber et al. (2001). **B:** Voltage responses of the interneuron model to 200-ms current injections of the same magnitude as in A. **C:** The model consists of 800 principal cells (red and green dots, 400 each, represent principal cells in LAdd and LAdv, respectively) and 200 interneurons (black dots). The principal cells in the model were populated randomly in the horn shaped tridimensional structure with dimensions of 800 μm in the rostral-caudal, 800 μm in the ventral-dorsal, and 400 μm in the medial-lateral directions. **D:** Fear conditioning protocol. As in the experiments of Repa et al. (2001), the "behavioral" protocol included habituation, conditioning and extinction phases, with 8, 16 and 20 trials, respectively.

Results

A. LP cell numbers remained relatively constant across various CREB cases. Cells receiving increased inhibition become TP cells.

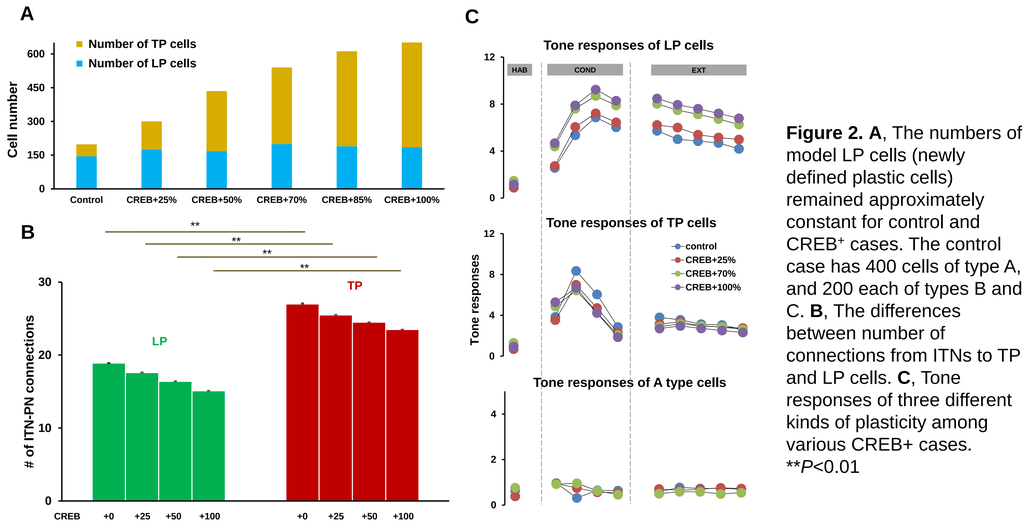

Figure 2. A, The numbers of model LP cells (newly defined plastic cells) remained approximately constant for control and CREB$^+$ cases. The control case has 400 cells of type A, and 200 each of types B and C. **B**, The differences between number of connections from ITNs to TP and LP cells. **C**, Tone responses of three different kinds of plasticity among various CREB+ cases. $^{**}P<0.01$

B. Plastic PNs band together to inhibit other PNs via disynaptic connections

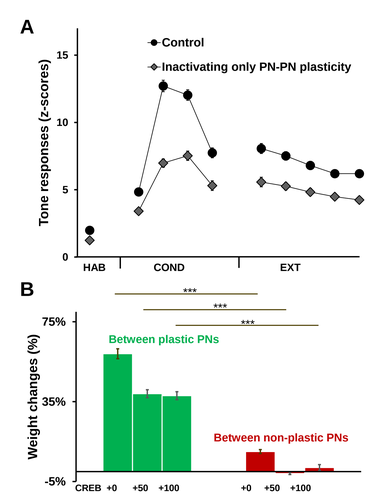

Figure 3. A, Average (±SEM) tone responses decreased significantly with inactivation of PN-PN plasticity. **B**, Weight changes (%) for PN-PN connections between plastic and non-plastic PNs.
$^{***}P<0.001$

Table 1. Differences between disynaptic inhibitory and excitatory connections (avg ±SEM) for winners and loser PN categories, from Winners+Losers as a group, for the CREB cases.

Characteristics of type B and C PNs		# of disyn INH-EXC connections(# of cells)		
		CREB+25%	CREB+50%	CREB+100%
Winners as post-synaptic cells	T&S, NE and DA	45±6 (24) p=0.0025	56±6 (22) p=0.0022	66±7 (34) p<0.001
	T&S, NE	49±8 (10) p=0.1871	59±7 (19) p=0.0452	77±7 (29) p=0.0583
	T&S, DA	44±7 (8) p=0.0815	56±9 (11) p=0.0207	71±10 (13) p=0.0016
	T&S, no modulators	61±9 (15) p=0.8075	61±7 (6) p=0.1658	76±14 (13) p=0.0613
	only T, NE and DA	--	42±2 (2) p=0.0414	42±13 (3) p<0.001
	only T, NE	--	76±16 (7)	108±16 (8) p=0.3381
	only T, DA	84±15 (2) p=0.2961	49±0 (1) p=0.0414	69±10 (4) p=0.236
	only T, no modulators	--	15±8 (3)	41±18 (4)
	only S, NE and DA	55±17 (4) p=0.6551	25±22 (2)	37±29 (2) p=0.6212
	only S, NE	--	42±0 (1)	53±0 (1)
	only S, DA	30±0 (1) p=0.1840	13±0 (1) p=0.1006	22±0 (1) p=0.1296
Characteristics of type B and C PNs		CREB+25%	CREB+50%	CREB+100%
Losers as post-synaptic cells	T&S, NE and DA	75±4 (12)	82±5 (22)	107±6 (33)
	T&S, NE	75±8 (2)	93±11 (4)	105±8 (8)
	T&S, DA	64±8 (6)	84±5 (10)	114±7 (12)
	T&S, no modulators	70±0 (1)	82±11 (2)	128±11 (4)
	only T, NE and DA	86±15 (5)	101±12 (6)	151±9 (6)
	only T, NE	46±10 (2)		57±0 (1)
	only T, DA	50±19 (2)	67±13 (3)	104±33 (2)
	only T, no modulators	--	--	--
	only S, NE and DA	36±0 (1)		71±0 (1)
	only S, NE			
	only S, DA	49±5 (3)	68±10 (4)	91±15 (4)
	none, DA	84±0 (1)	91±0 (1)	130±0 (1)

Discussion & Conclusion

A.
- Model runs of 25 to 100% CREB over-expression cases revealed that while the numbers of LP cells remained constant across these cases, those of TP cells varied widely. This suggests that LP cells form the 'fear memory trace' reported in Han et al. ('07,'09).
- Mechanism of formation of TP cells? Although TP cells received similar numbers of excitatory connections as LP cells, they received significantly higher inhibition compared to LP cells, reducing their tone responses to habituation levels by the end of conditioning (Fig 2B).

B.
- Compared to the control case, fear conditioning in the absence of PN-PN plasticity produced a large decrease in CS responsiveness during conditioning (Fig. 3A). This effect was paralleled by a significant decrease in the number of plastic PNs, from 211 to 151.
- Model runs suggest that conditioning-induced strengthening of connections (Fig. 3B) enable plastic PNs to band together effectively to inhibit other PNs via disynaptic connections involving interneurons.
- Disynaptic inhibition from winners+losers as a group was lower for winners than losers for PNs. The presence of tone, shock, and NM receptors were also the attributes that increased the chances of a PN becoming a winner in the competition (Table 1).

Future Work: A recent report (Wolff et al., 2014) suggests that the LA cells are dis-inhibited during shock. We will revise the 1000-cell biophysical model to incorporate this new finding (Fig 4), and use it to explore neural correlates of competition.

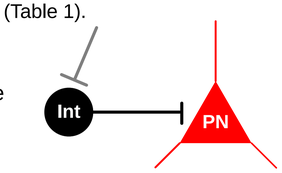

Fig 4. LA interneurons are inhibited during unconditioned stimulus (US).

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Acknowledgments

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